

(19) World Intellectual Property
Organization
International Bureau



Rec'd PCT/PTO

23 MAR 2005



(43) International Publication Date
8 April 2004 (08.04.2004)

PCT

(10) International Publication Number
WO 2004/028276 A3

- (51) International Patent Classification⁷: **A23L 1/105**, 1/03, A21D 8/04, A23L 1/217
- (21) International Application Number: PCT/GB2003/004046
- (22) International Filing Date: 24 September 2003 (24.09.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 0222185.1 24 September 2002 (24.09.2002) GB
- (71) Applicant (for all designated States except US): **FORINNOVA AS** [NO/NO]; Thormøhlensgate 55, N-5008 Bergen (NO).
- (71) Applicant (for GB only): **COCKBAIN, Julian** [GB/GB]; 42 Southmoor Road, Oxford OX2 6RD (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **BAARDSETH, Pernille** [NO/NO]; Forinnova AS, Thormøhlensgate 55, N-5008 Bergen (NO). **BLOM, Hans** [NO/NO]; Forinnova AS, Thormøhlensgate 55, N-5008 Bergen (NO). **ENERSEN, Grethe** [NO/NO]; Forinnova AS, Thormøhlensgate 55, N-5008 Bergen (NO). **SKREDE, Grete** [NO/NO]; Forinnova AS, Thormøhlensgate 55, N-5008 Bergen (NO). **SLINDE, Erik** [NO/NO]; Forinnova AS, Thormøhlensgate 55, N-5008 Bergen (NO). **SUNDT, Thea** [NO/NO]; Forinnova AS, Thormøhlensgate 55, N-5008 Bergen (NO). **THOMASSEN, Trond** [NO/NO]; Forinnova AS, Thormøhlensgate 55, N-5008 Bergen (NO).
- (74) Agents: **COCKBAIN, Julian** et al.; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).
- (81) Designated States (*national*): AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, EG, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK (utility model), SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- with international search report
 - before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 3 June 2004
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

BEST AVAILABLE COPY

(54) Title: REDUCTION OF ACRYLAMIDE FORMATION IN CEREAL-BASED FOOD PROCESSING

(57) Abstract: The invention relates to the use of acid treatment of carbohydrate-containing cereal-based foodstuffs or components prior to cooking by roasting, frying, grilling or baking whereby to reduce the acrylamide content of the cooked product.

WO 2004/028276 A3

- 1 -

Reduction of acrylamide formation in cereal-based food processing

5 The present invention relates to improvements in and relating to cooked food, in particular cereal-based foods which are fried, grilled, baked or roasted.

 In a publication by the Swedish National Food Administration (see www.slv.se/engdefault.asp) it was
10 reported that many cooked foods, in particular fried, grilled or baked foods, had surprisingly been found to contain high levels of the toxic contaminant acrylamide. No suggestion was made as to how the acrylamide context of such foods could be reduced.

15 A further report of acrylamide production in food cooking occurred in Tareke et al., J. Agric. Food Chem 50: 4998-5006 (2002).

 We have now surprisingly found that the acrylamide content of cooked cereal-based foods can be reduced by
20 treatment of the food or the cereal base therefor prior to cooking with lactic acid generating microorganisms and/or with acid.

 Thus viewed from one aspect the invention provides the use of a lactic acid producing microorganism for the
25 treatment of a cereal-based food material or the cereal base therefor to reduce acrylamide production in subsequent cooking thereof.

 Lactic acid producing microorganisms are well known and examples include lactic acid bacteria such as
30 Bifidobacterium sp., Brevibacterium sp., Lactobacillus sp., Lactococcus sp., Leuconostoc sp., Micrococcus sp., Oenococcus sp., Pediococcus sp., and Streptococcus sp. Lactobacilli are especially preferred for use according to the invention, in particular Lactobacillus plantarum
35 strains NCDO 1752 and NCDO 1193 (available from the National Collection of Food Bacteria) and Lactobacillus NCIMB 40450. Other strains of lactobacillus which

- 2 -

generate lactic acid and are safe for use in foodstuff treatment have been described widely in the scientific literature.

5 The treatment with a lactic acid producing microorganism according to the invention preferably involves incubation in an aqueous medium for up to 7 days, e.g. 30 minutes to 24 hours, especially 1 to 6 hours. Incubation is preferably at 4 to 45°C, e.g. 25 to 35°C, i.e. as is conventional for such
10 microorganisms.

Typically such treatment may involve homofermentative lactic acid bacteria incubation in an aqueous medium.

15 Viewed from a further aspect the invention provides the use of a physiologically acceptable acid for the treatment of a cereal-based food material or the cereal base therefor to reduce acrylamide production in subsequent cooking thereof.

20 The physiologically tolerable acid used according to the invention may be any acid acceptable for use in foodstuffs, e.g. organic acids, such as citric, malic, acetic, maleic, tartaric, succinic and lactic acids or inorganic acids such as hydrochloric, sulphuric and phosphoric acids and sulphur dioxide. The use of citric
25 and hydrochloric acids is especially preferred, as is the use of lactic acid and/or of phosphoric acid. The use of hydrochloric acid and of lactic acid is particularly preferred. The acid is preferably used in a quantity and strength sufficient to reduce the surface
30 pH of the food material treated to 1 to 5.5, preferably 3 to 5, especially about 4. Following acid treatment, the food material is preferably stored for up to 7 days (e.g. 30 minutes to 24 hours, especially 1 to 6 hours before cooking or freezing.

35 In this process, the acid is preferably used in the form of a buffer solution.

Following treatment with the acid and/or the lactic

- 3 -

acid producing microorganism, the food material may be cooked using cooking techniques that expose the material to temperatures above 150°C, e.g. by baking, grilling, roasting or frying.

5 Before such high temperature cooking, it is desirable to rinse the treated food material with water.

 The cooking may be a single stage operation. However it may instead be one stage of a multi stage (e.g. two stage) cooking procedure. Thus the technique
10 of the invention is especially applicable to food materials which are treated according to the invention, partially cooked, transported and/or stored, then cooked again.

 The invention is particularly applicable to
15 products made from cereal (e.g. rice, barley, wheat, rye, oat, maize, etc.) flours, granulates or fragments, in particular breads (especially crisp-breads, biscuits, wafers, cookies and crackers), cakes, snacks (e.g. crisps (in American-English chips), pretzels, and the
20 like), and breakfast cereals (e.g. "cornflakes" and the like).

 Thus in a further aspect the invention provides a process for the production of a food product which comprises fermenting a granulated or crushed
25 carbohydrate-containing cereal material with a lactic acid producing microorganism, optionally formulating the fermented material into a shaped product (e.g. by extrusion, rolling or moulding a paste or dough), and cooking to produce said food product.

30 In place of fermentation, acid treatment as described above may be used: however this is less preferable.

 The granulated carbohydrate-containing cereal material, e.g. a cereal flour, may be mixed with un-
35 treated granulated carbohydrate-containing cereal material before cooking. Desirably the treated:untreated weight ratio is from 25:75 to 100:0,

- 4 -

especially 50:50 to 95:5.

The term granulate or granulated as used herein may where the context permits include fine to coarse particulates, e.g. flours, granules, grits, fragments, etc. Preferably however the granulates will be 2mm or smaller in maximum dimension.

The food products thus produced are desirably packed into sealed, preferably sterilized containers, e.g. cartons, plastic or foil bags, bottles, etc. Particularly preferably such containers are moisture-proof.

Where appropriate, the food products produced according to the invention may be further processed, e.g. by drying, freezing, cutting, decorating, etc. Such processing steps, which are often conventional for the particular food product, form further optional steps in the processes of the invention.

French-fries may also be made from extruded or moulded carbohydrate-containing pastes produced using powdered or granulated cereal (e.g. rice) optionally together with powdered or granulated potato.

The invention is applicable to breads, biscuits (known in America as cookies), and in particular crisp breads. In this aspect of the invention, the treatment according to the invention may be effected using lactic acid bacteria in the production of the dough and/or by acid treatment (e.g. treatment with sulphur dioxide or hydrogen chloride) of the flour.

Besides the fermentation and/or acid treatment according to the invention, the food products of the invention may be prepared by conventional methods, optionally involving rinsing and/or drying after the treatment. Thus such food products may optionally contain further components, such as conventional foodstuff components or additives, e.g. salt, sugars, flavours, fruit, fruit extracts, nuts, eggs, milk, flour, bread, breadcrumbs, stabilizers, colours,

- 5 -

buffers, acidulants, yeast, bicarbonate, etc.

The invention will now be illustrated further with reference to the following non-limiting Example.

5 Example 1

Crisp bread

Ingredients

Finely ground whole rye flour was obtained from Cerealia Mills, Oslo, Norway.

10

Crisp bread recipe

The crisp bread dough was based on a standard recipe made from 1150g rye flour, 1000g water, 20g NaCl. The ingredients were mixed and the dough was rolled out to proper thickness and baked in a stone oven at 240°C.

15

Acid equivalent

Total amount of acid in the flour-water mixtures was determined as Acid equivalents, S°, by titration. To 10g flour-water mixture, 90g of distilled water is added. During stirring, 0.1 N NaOH is added until a stable pH of 8.5 is reached. Acid equivalents is expressed as the amount of 0.1 N NaOH consumed, in ml.

20

25 Pre-treatments

Fermentation

The rye flour used in the crisp bread dough was fermented using a lactic acid bacteria (e.g. NCIMB 40450 or others described herein) according to the following description: 1000g rye flour and 1000g tap water at 30°C was mixed and bacteria (10^6 bacteria/g flour) was added. The bacteria had previously been cultured in MRS growth medium, harvested in the exponential phase, centrifuged and dispersed in water, prior to being added to the flour. Flour fermentation was performed at 30°C in a proofing cabinet at 70% relative humidity (RH) for about 18 hours.

30

35

- 6 -

Soaking in lactic acid

The rye flour used in the crisp bread was soaked in lactic acid according to the following description:
1000g flour was mixed with 1000g 0.15 M lactic acid at
30°C.

5

Soaking in acidic phosphate buffer

The rye flour used in the rye crisp bread was soaked in phosphate buffer according to the following description:
1000g flour was mixed with 1000g 0.1 M phosphate buffer,
pH 4.0, 30°C.

10

Doughs with the following composition were produced from the pre-treated or non-pre-treated flours:

15

1. No pre-treatment

345g rye flour + 300g water + 7g NaCl was mixed for 4 min in a Hobardt mixer equipped with a dough hook.

20

2. Fermentation - lactic acid bacteria added

450g fermented flour/water mixture (bacteria added) + 150g untreated rye flour + 7g NaCl was mixed for 4 min in a Hobardt mixer equipped with a dough hook.

25

3. Lactic acid

450g flour/water mixture in lactic acid + 150g untreated rye flour + 7g NaCl was mixed for 4 min in a Hobardt mixture equipped with a dough hook.

30

4. Phosphate buffer

450g flour/water mixture in phosphate buffer + 150g untreated rye flour + 7g NaCl was mixed for 4 min in a Hobardt mixer equipped with a dough hook.

35

The proportion of pre-treated flour in doughs 2 to 4 was thus 60%.

- 7 -

About 100g of dough was rolled to 0.5mm thickness and baked at 240°C for 10 min.

Analysis of acrylamide was effected by Norsk Matanalyse AS, Oslo, Norway.

Results

The results are presented in Table 1 below.

	Pre-treatment of rye flour	pH after pre- treatment	Acid equivalent		Acrylamide
			ml 0.1N NaOH/10g	µg/kg product (ppb)	% reduction*
10	No-pretreatment	6.00	4.0	3500	-
15	Fermentation - lactic acid bacteria added	3.70	18	890	75
20	Soaked in lactic acid	4.05	20	1000	71
	Soaked in phosphate buffer	4.74	11	1400	60

*Reduction relative to crisp bread with no pre-treatment.

The pH in the rye flour/water mixture with bacteria added decreased during the pre-treatment. This shows that fermentation had occurred with corresponding acid production. The pH of the mixture with lactic acid and phosphate buffer added was as expected. At the applied conditions, adding lactic acid was effective in lowering pH and was thus similar to the controlled fermentation. The acid equivalent values reflected the changes seen in pH, with higher levels corresponding to the most extensive changes in pH.

Acrylamide levels in crisp bread reflected the various

- 8 -

- pre-treatments tested. With no pre-treatment, the acrylamide level was high. The use of 60% rye flour fermented by the adding of lactic acid bacteria caused a 75% reduction in acrylamide compared with crisp bread produced without pre-treatment. The reduction was 71% when 60% of the flour was soaked in lactic acid as a pre-treatment. With phosphate buffer, the reduction in acrylamide was 60%.
- 10 It is likely that the pre-treatment can be optimised in several ways. First, the present experiments were performed with pre-treatment of only 60% of the total flour in the crisp bread. Doughs based on 100%-fermented flour may be used. Alternatively, the
- 15 fermented flour may be fully or partly dried prior to application in the crisp bread dough, thus making it possible to adjust the viscosity of the dough to a level appropriate for rolling. Combinations of fermentation and acids may be effective in further lowering
- 20 acrylamide levels, as well as sole adding of lactic or other acids to higher levels, and/or lower pH values.

- 9 -

Claims:

1. The use of a lactic acid producing microorganism for the treatment of a cereal-based food material or the cereal base therefor to reduce acrylamide production in subsequent cooking thereof.
2. Use as claimed in claim 1 of a lactic acid bacterium.
3. The use of a physiologically acceptable acid for the treatment of a cereal-based food material or the cereal base therefor to reduce acrylamide production in subsequent cooking thereof.
4. Use as claimed in claim 3 of an acid selected from lactic, citric, phosphoric and hydrochloric acids.
5. A process for the production of a food product which comprises fermenting a granulated or crushed carbohydrate-containing cereal material with a lactic acid producing microorganism, optionally formulating the fermented material into a shaped product, and cooking to produce said food product.
6. A process for the production of a food product which comprises treating a granulated or crushed carbohydrate-containing cereal material with a physiologically tolerable aqueous acid, optionally formulating the treated material into a shaped product, and cooking to produce said food product.
7. A process as claimed in either of claims 5 and 6 further comprising packaging the food product into sealed sterilized containers.
8. A cereal-based food material produced by a process

- 10 -

as claimed in any one of claims 5 to 7 or by a use as
claimed in any one of claims 1 to 4.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.